Toxicity and sublethal effects of six insecticides to last instar larvae and adults of the biocontrol agents *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae)

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A B S T R A C T

To further develop Integrated Pest Management (IPM) strategies against crop pests, it is important to evaluate the effects of insecticides on biological control agents. Therefore, we tested the toxicity and sublethal effects (fecundity and fertility) of flonicamid, flubendiamide, metaflumizone, spirotetramat, sulfoxaflor and deltamethrin on the natural enemies *Chrysoperla carnea* and *Adalia bipunctata*. The side effects of the active ingredients of the insecticides were evaluated with residual contact tests for the larvae and adults of these predators in the laboratory. Flonicamid, flubendiamide, metaflumizone and spirotetramat were innocuous to last instar larvae and adults of *C. carnea* and *A. bipunctata*. Sulfoxaflor was slightly toxic to adults of *C. carnea* and was highly toxic to the L_4 larvae of *A. bipunctata*. For *A. bipunctata*, sulfoxaflor and deltamethrin were the most damaging compounds with a cumulative larval mortality of 100%. Deltamethrin was also the most toxic compound to larvae and adults of *C. carnea*. In accordance with the results obtained, the compounds flonicamid, flubendiamide, metaflumizone and spirotetramat might be incorporated into IPM programs in combination with these natural enemies for the control of particular greenhouse pests. Nevertheless, the use of sulfoxaflor and deltamethrin in IPM strategies should be taken into consideration when releasing either of these biological control agents, due to the toxic behavior observed under laboratory conditions. The need for developing sustainable approaches to combine the use of these insecticides and natural enemies within an IPM framework is discussed.

1. Introduction

The impact of synthetic pesticides on the environment, the beneficial arthropods and the human health by exposure to these chemicals are issues of growing concern. In response to this, the European Union Directive 2009/128/EC established a legal framework to achieve the sustainable use of pesticides and to implement Integrated Pest Management (IPM) strategies in all member states by 1st January 2014 (EEC/CEE, 2009). The priority of IPM is the use of biological control, but biocontrol may not always be effective enough to manage insect pest populations, and corrective insecticide treatments may be needed, particularly in greenhouses, where the incidence of pests is higher (Medina et al., 2008; Jalali et al., 2009). Chemical control may be required to suppress pests that have no efficient biological control agents,

and thus the identification of products that are not harmful to beneficial organisms and are respectful of the environment is a primary concern in IPM programs (Viñuela et al., 2000; Hassan and Van Veire, 2004). The main premise for the use of pesticides in IPM systems is to use products with proven selectiveness to biological control agents (BCAs), low mammalian and avian toxicity, minimal environmental persistence, and low risk of developing resistance in the target populations, yet with a fairly broad spectrum of insecticidal activity against pests (Van Lenteren and Woets, 1988; Harris, 2000; Viñuela et al., 2000). An accurate evaluation of the potential side effects of insecticides on BCAs is critical for developing effective IPM strategies (Desneux et al., 2006; Stark et al., 2007).

The green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) is found in a wide range of agricultural habitats and ranks as one of the most commonly used and commercially available natural enemies (Tauber et al., 2000; New, 2001). The lacewing is considered a key generalist biological control agent, which is used primarily through augmentative periodic releases of larvae for the control of various aphid species in greenhouses and outdoor crops (Medina et al., 2002; Van der Blom, 2008; Turquet et al., 2009). However, *C. carnea* larvae also consume a wide variety of soft-bodied arthropods, such as scales, leafhoppers, whiteflies, psyllids, thrips, eggs and larvae of lepidopterans, and mites (Principi and Canard, 1984; Rimoldi et al., 2008).

The ladybird *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) is commercially available and widely used in Spain to protect horticultural crops (Robledo Camacho et al., 2009). Its diet is composed of aphids, which are consumed both by larvae and adults (Hodek and Honěk, 1996). The contribution of coccinellids to decreased population growth rates of aphids is well known (Hodek and Honěk, 1996; Volkl et al., 2007).

Amongst phytophagous insects, aphids, thrips and whiteflies are the most serious pests of greenhouse crops (Rabasse and Van Steenis, 1999). These insects cause mechanical damage with their sucking activity and transmit numerous plant viruses (Ng and Perry, 2004). Despite outstanding characteristics as aphid predators, *C. carnea* and *A. bipunctata* do not always maintain populations of aphid pests below economic thresholds, and thus insecticides remain an important management tool in greenhouse IPM programmes to control key pests (Desneux et al., 2007; Obrycki et al., 2009). To generate the IPM guidelines for natural enemy conservation requires not only the assessment of insecticide lethal effects but also the possible sublethal effects in individuals who survive an exposure to a particular product (Desneux et al., 2007; Moscardini et al., 2013) because these effects could have an important effect on natural enemy population dynamics (Stark and Banks, 2003).

Therefore, the objective of this study was to evaluate the lethal and sublethal effects of six pesticides: deltamethrin, flonicamid, flubendiamide, metaflumizone, spirotetramat (included in the Phytosanitary Products Registry of the Spanish Ministry of Agriculture, Food and Environment; MAGRAMA, 2014), and sulfoxaflor. *C. carnea* and *A. bipunctata* larvae and adults were tested in the laboratory to classify the toxicity of the insecticides in accordance with the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) directives.

2. Materials and methods

The bioassays were conducted in the Laboratory of Crop Protection, Department of Agrarian Production, Technical University of Madrid, Spain. The laboratory conditions were controlled at 25 ± 2 °C, with relative humidity at 75 ± 10%, and a photoperiod of 16:8 (light:dark).

2.1. Insects

A laboratory colony of *C. carnea* was established with L_1 larvae obtained from Agrobío (CHRYSOcontrol®, Almería, Spain) following the rearing procedure of Medina et al. (2001). The larvae were fed *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs *ad libitum*, and adults were provided a nutritious artificial diet (Vogt et al., 2000). For the trials, gauze with <24-h-old eggs was used and let to develop the L_3 instar for the larvae bioassays and the adults (<48-h post emergence) for imaginal bioassays.

A. bipunctata L_1 larvae were purchased from Agrobío (ADALIAcontrol®, Almería, Spain) and were placed in cages with zigzag folded filter paper and a mixture of *E. kuehniella* eggs and bee pollen (1:1) replaced three times per week (Bonte et al., 2010) up to the L_4 moult; the fourth larval instar was used for testing the insecticides for side effects. For the adult experiments, L_4 larvae were placed in individual arenas with a small amount of diet to avoid cannibalism and to guarantee successful pupation. After 6–7 d, the newly emerged adults were transferred to cages with a water source (baize soaked with water) and the same diet provided to larvae. *A. bipunctata* adults, <48-h from emergence, were used in the imaginal experiments.

2.2. Insecticides

In the experiments, six commercial formulations of insecticides were tested. The active ingredients that were evaluated are currently used in IPM programs on protected crops of southeastern Spain, except for sulfoxaflor, which is a product under evaluation process. All compounds were tested at the maximum field recommended concentrations for horticultural crops, according to the product labels and following the instructions provided by the Spanish Ministry of Agriculture, Food and Environment. The insecticides, concentrations of active ingredient tested, and modes of action (IRAC, 2012; MAGRAMA, 2014) were as follows: flonicamid 60 mg a.i. L^{-1}, a selective homopteran feeding blocker (Teppeki® 50% w/w water dispersible granules; Ishihara Sangyo Kaisha Ltd., Osaka, Japan); flubendiamide 60 mg a.i. L^{-1}, a ryanodine receptor modulator (Fenos® 24% w/w water dispersible granules; Bayer S.A.S., Lyon, France); metaflumizone 240 mg a.i. L^{-1}, a voltage-dependent sodium channel blocker (Alverde® 24% w/v suspension concentrate; BASF SE, Ludwigshafen, Germany); spirotetramat 75 mg a.i. L^{-1}, an inhibitor of acetyl CoA carboxylase (Movento® 150 O-TEC 15% w/v oil dispersion; Bayer CropScience AG, Monheim am Rhein, Germany); sulfoxaflor 63.6 mg a.i. L^{-1}, a nicotinic acetylcholine receptor (nAChR) agonist (GF-2626 11.43% w/w suspension concentrate; Dow AgroSciences, Indianapolis, IN, US); and deltamethrin 12.45 mg a.i. L^{-1}, a sodium channel modulator (Decis® EW 15 1.5% w/v emulsion in water; Bayer CropScience S.L., Paterna, Valencia, Spain), which is used as a broad spectrum insecticide in horticultural crops.

2.3. Bioassays

Residual contact experiments were conducted because this is the primary way the larvae and adults of both biocontrol agents are exposed to insecticide contamination. Glass plates (12 × 12 × 0.5 cm) were sprayed with a Potter precision spray tower (Burkard Manufacturing Co., UK) to test the residual contact activity of the pesticides. The standard application was between 1.5 and 2 mg cm^{-2} (1 ml at 55 kPa); this is the concentration interval recommended by the IOBC validity criteria for ecotoxicological experiments on beneficial arthropods (Hassan, 1994). As soon as the glass plates were dry, larvae and adults were exposed to the insecticides with two different methods.

2.3.1. Larvae bioassays

The larvae bioassays used seven individualized L_3 or L_4 (for *C. carnea* and *A. bipunctata*, respectively) larvae <48-h from moulting per replicate and 6 replicates per treatment, following the procedure of Medina et al. (2004). Larval mortality, percentage of pupae formed and successful adult emergence from those pupae were recorded. Cumulative mortality was the percentage of individuals who failed to reach the complete adult form. All the adults that emerged from the same treatment were collected and placed together until the beginning of oviposition.

For the *C. carnea* assays, as many replicates as possible of three pairs of adults (depending on the survivorship: control, 7; flonicamid, flubendiamide, metaflumizone and spirotetramat, 6; sulfoxaflor, 5; deltamethrin, 3) were maintained in plastic oviposition cages (12 cm in diameter × 5 cm in height) and provided food and water to assess the reproductive parameters according to Vogt et al. (2000). A piece of cotton gauze was placed in the upper part of the oviposition cages, where the eggs of *C. carnea* were collected to measure fecundity. The reproduction test started 5–7 d after the first egg was laid. Along one week, two samples of <24-h eggs were collected from each replicate and kept in separate plastic cages (9 cm in diameter × 2 cm in height) for incubation until hatching, and the egg viability was determined (Giolo et al., 2009).

For *A. bipunctata*, because of the scarce morphological evidence of sexual dimorphism (Hodek and Honěk, 1996), the adults that emerged were divided into as many groups of 10 individuals each as possible (control, flonicamid, flubendiamide, metaflumizone and spirotetramat, 4; sulfoxaflor and deltamethrin, 0), and placed into plastic oviposition cages (12 cm in diameter × 5 cm in height), providing food and water (*E. kuehniella* eggs and bee pollen, 1:1; Bonte et al., 2010) to assess the reproductive parameters. In the upper part of the oviposition cages was placed a cotton gauze and inside them wrinkled filter paper, where the eggs of *A. bipunctata* were collected. The reproduction tests began 4–5 d after adult emergence. The eggs were sampled and treated as described for *C. carnea*, but the evaluation was conducted for two weeks. The individuals of each group were sexed *a posteriori* (by dissection and extraction of the genitalia) to determine the sex ratio and the number of eggs laid per female.

2.3.2. Adults bioassays

For bioassay with the adults of *C. carnea*, individuals (<48-h from emergence) were exposed to dried residues on the glass surfaces. Three pairs (3 ♂ and 3 ♀) of adults were a replicate, and each treatment had five replicates. The test units were described by Giolo et al. (2009) and were used as soon as the plates were dry. The mortality was recorded after 3 d of residual contact. After the residual contact, the surviving adults were transferred to the plastic cages described above to evaluate reproduction, maintaining the initial number of replicates (5) for each treatment. The effects on reproductive parameters (fecundity and fertility) were determined as described for the larvae bioassay.

In the bioassay with *A. bipunctata* adults, 14 individuals (<48 h from emergence) per replicate were exposed to dried residues on the glass surfaces, and each treatment had five replicates. The test units were the same as in the experiment with *C. carnea*, and the performance of the assay and the evaluation process were identical. The initial number of replicates per treatment (5) was maintained to assess the reproductive parameters. The surviving individuals of each replicate were sexed *a posteriori* to evaluate the reproductive parameters.

2.4. Data analyses

Data, presented as the means ± SE, were analyzed using one-way analysis of variance (ANOVA) with the Statgraphics Plus 5.1 statistical software package (Statistical Graphics Corp., 1994–2000). The means were separated using the Fisher least significant difference (LSD) multiple range test ($P < 0.05$). The nonparametric tests of Kruskal–Wallis and Mann–Whitney ($P < 0.05$) were used to establish differences only when data violated the premises of the ANOVA (Heath, 1995).

The total effect (E) of each insecticide was determined taking into account the percentage of mortality observed in each treatment in relation to the mortality observed in the control treatment, and corrected using the Schneider–Orelli's formula (Püntener, 1981). It was used the formula proposed by Overmeer and Van Zon (1982): $E\ (\%) = 100 - (100 - Mc) \times ER$; where Mc is the final corrected mortality and ER is the ratio of mean number of eggs laid weekly by treated females versus control females.

According to the IOBC laboratory scale (Hassan, 1994) and based on their total effects, the pesticides were classified in four toxicity categories: (1) harmless (<30%); (2) slightly harmful (30–79%); (3) moderately harmful (80–99%); and (4) harmful (>99%).

3. Results

3.1. Effects of insecticides on C. carnea

Significant differences were found in the L_3 larvae bioassay among treatments in pupation ($F_{6,35} = 14.49$; $P < 0.001$) and cumulative mortality ($F_{6,35} = 12.39$; $P < 0.001$), in particular for deltamethrin compared with the control. Regarding adult emergence, the results did not show significant differences ($H = 9.61$; $P = 0.142$). No statistically significant differences were found for fecundity among treatments ($F_{6,32} = 0.42$; $P = 0.863$). For fertility of eggs laid by females significant differences were obtained ($F_{6,32} = 2.68$; $P = 0.032$), among the insecticides flubendiamide and sulfoxaflor compared with the control treatment (Table 1).

When testing the insecticides on adults, only sulfoxaflor and deltamethrin had significant effects on the mortality at 3 d compared with the control ($F_{6,28} = 17.49$; $P < 0.001$). Statistically significant differences were found between these two insecticides as well, with the mortality (at 3 d) of sulfoxaflor reaching 56.67% (Table 2). For the reproductive parameters (eggs/♀ week^{-1} and viable eggs laid), only deltamethrin significantly decreased fecundity (15.20 eggs/♀ week^{-1}) ($F_{6,28} = 8.72$; $P < 0.001$) and fertility (3.54%) ($F_{6,26} = 16.21$; $P < 0.001$) compared with the rest of the treatments (Table 2).

Flonicamid, flubendiamide, metaflumizone and spirotetramat were harmless (IOBC class 1) to L_3 larvae and adults of *C. carnea*. Sulfoxaflor proved to be harmless (IOBC class 1) only to L_3 larvae of *C. carnea*, whereas it was slightly toxic (IOBC class 2) to *C. carnea* adults. Deltamethrin was slightly toxic (IOBC class 2) and moderately toxic (IOBC class 3) to L_3 larvae and adults of *C. carnea* respectively.

3.2. Effects of insecticides on A. bipunctata

In the *A. bipunctata* larvae bioassay, sulfoxaflor and deltamethrin were much more detrimental than with *C. carnea*. Statistically significant differences were found for pupation ($H = 38.62$; $P < 0.001$), adult emergence ($H = 27.34$; $P < 0.001$) and cumulative mortality ($H = 36.76$; $P < 0.001$) between these two compounds and the rest of treatments, including the control. Sulfoxaflor reduced significantly the percentage of pupation (7.14%), and furthermore, of those scarce pupae that formed, no adults emerged. The effects of deltamethrin were stronger than sulfoxaflor because no pupae were formed and every larva died (Table 3). The reproductive parameters were not significantly different (fecundity: $F_{4,15} = 2.45$; $P = 0.092$; fertility: $F_{4,15} = 1.62$;

Table 1
Effects on development, mortality and reproduction in *Chrysoperla carnea* when L_3 larvae were exposed to fresh residues of insecticides on a glass surface, using the maximum field recommended concentration (MFRC) for horticultural crops.

Treatment	MFRC (mg a.i. L^{-1})	Pupation (%)[a]	Adult emergence (%)[b]	Cumulative mortality (%)[c]	Fecundity (eggs/♀ $week^{-1}$)[d]	Fertility (%)[e]	*E* (IOBC Cat.)[f]
Control	–	100.00 ± 0.00 a	100.00 ± 0.00 a	0.00 ± 0.00 a	217.88 ± 17.80 a	76.94 ± 1.98 a	–
Flonicamid	60	100.00 ± 0.00 a	95.24 ± 3.01 a	4.76 ± 3.01 a	190.55 ± 16.80 a	75.39 ± 2.91 a	16.69 (1)
Flubendiamide	60	92.85 ± 3.19 a	94.44 ± 5.55 a	11.91 ± 6.82 a	204.06 ± 29.27 a	66.22 ± 3.50 b	17.49 (1)
Metaflumizone	240	97.62 ± 2.38 a	100.00 ± 0.00 a	2.38 ± 2.38 a	207.83 ± 26.91 a	71.80 ± 1.25 ab	6.90 (1)
Spirotetramat	75	90.47 ± 7.06 a	100.00 ± 0.00 a	9.52 ± 7.06 a	203.72 ± 20.49 a	71.68 ± 1.91 ab	15.42 (1)
Sulfoxaflor	63.6	90.47 ± 3.01 a	97.22 ± 2.78 a	11.91 ± 4.39 a	225.00 ± 22.89 a	67.78 ± 1.64 b	9.04 (1)
Deltamethrin	12.45	54.76 ± 6.82 b	86.11 ± 6.69 a	52.38 ± 7.06 b	241.22 ± 8.47 a	70.67 ± 3.30 ab	47.29 (2)

Data (mean ± SE) followed by different letters in the same column significantly differed (ANOVA, LSD, $P < 0.05$) for pupation, cumulative mortality, fecundity and fertility.
Data (mean ± SE) followed by the same letter did not significantly differ (Kruskal–Wallis, $P < 0.05$) for adult emergence.
a.i. = active ingredient. *E* = total effect, according to Overmeer and Van Zon (1982).
[a] Percentage of formed pupae compared with the total number of treated larvae.
[b] Percentage of emerged adults compared with the number of formed pupae.
[c] Percentage of dead larvae, pupae and adults that failed to moult.
[d] Number of eggs laid per female measured 5–7 d after the first egg laying.
[e] Percentage of hatched eggs versus laid eggs.
[f] IOBC toxicity category for laboratory tests based on the total effect caused by each insecticide: (1) harmless (<30%); (2) slightly harmful (30–79%); (3) moderately harmful (80–99%); and (4) harmful (>99%).

Table 2
Effects on mortality and reproduction in *Chrysoperla carnea* when adults were exposed to fresh residues of insecticides on a glass surface, using the maximum field recommended concentration (MFRC) for horticultural crops.

Treatment	MFRC (mg a.i. L^{-1})	Adult mortality at 3 d (%)	Fecundity (eggs/♀ $week^{-1}$)[a]	Fertility (%)[b]	*E* (IOBC Cat.)[c]
Control	–	0.00 ± 0.00 a	253.70 ± 12.40 a	71.03 ± 1.40 a	–
Flonicamid	60	6.67 ± 4.08 ab	259.20 ± 20.26 a	70.06 ± 1.20 a	4.65 (1)
Flubendiamide	60	0.00 ± 0.00 a	227.40 ± 17.21 a	73.04 ± 2.99 a	10.37 (1)
Metaflumizone	240	13.34 ± 3.33 ab	245.60 ± 11.61 a	73.44 ± 2.59 a	16.10 (1)
Spirotetramat	75	0.00 ± 0.00 a	227.00 ± 15.90 a	66.49 ± 5.61 a	10.52 (1)
Sulfoxaflor	63.6	56.67 ± 8.50 c	184.80 ± 67.18 a	61.55 ± 16.10 a	68.44 (2)
Deltamethrin	12.45	20.00 ± 8.16 b	15.20 ± 10.16 b	3.54 ± 2.40 b	95.21 (3)

Data (mean ± SE) followed by different letters in the same column significantly differed (ANOVA, LSD, $P < 0.05$).
a.i. = active ingredient. *E* = total effect, according to Overmeer and Van Zon (1982).
[a] Number of eggs laid per female measured 5–7 d after the first egg laying.
[b] Percentage of hatched eggs versus laid eggs.
[c] IOBC toxicity category for laboratory tests based on the total effect caused by each insecticide: (1) harmless (<30%); (2) slightly harmful (30–79%); (3) moderately harmful (80–99%); and (4) harmful (>99%).

$P = 0.221$). No significant differences were found for fertility of eggs, and values of all treatments were below 60% (Table 3).

The results with adults showed a similar scenario; mortality at 3 d after treatment was significantly higher than the control for sulfoxaflor (22.86%) and deltamethrin (100.00%; Table 4) ($F_{6,28} = 55.72$; $P < 0.001$). The treatments did not significantly differ for fecundity ($F_{5,23} = 0.42$; $P = 0.828$) and viability of the eggs ($F_{5,23} = 1.98$; $P = 0.120$). In the adults bioassay, the overall fecundity reached higher values than those of the larvae assay, but all were less than 70% (Table 4).

As occurred with *C. carnea*, flonicamid, flubendiamide, metaflumizone and spirotetramat were harmless (IOBC class 1) to L_4 larvae and adults of *A. bipunctata*. Sulfoxaflor was harmless (IOBC class 1) just to adults of *A. bipunctata*, while it was toxic (IOBC class 4) to L_4 larvae. Deltamethrin was toxic (IOBC class 4) to both L_4 larvae and adult stages of the coccinellid.

4. Discussion

Among the six compounds evaluated, only deltamethrin significantly affected the pupation and cumulative larval mortality of *C. carnea*, in the L_3 bioassay. The toxic effect of deltamethrin by residual contact was also observed in the L_1 larvae of *C. carnea* by Bigler and Waldburger (1994), although other authors found innocuous effects of this insecticide to first instar larvae and adults following exposure (Giolo et al., 2009). Larvae of predators can be more easily damaged by pesticides than adults because they walk on treated surfaces and cannot fly, but some exceptions have been reported in the case of *C. carnea*, that seems to be more resistant in the larval stage (Medina et al., 2004). Despite its slightly toxic effect on L_3 larvae, this developmental stage showed less susceptibility than the adult one. Ishaaya and Casida (1981) reported that esterases present in the larvae of *C. carnea* have high hydrolyzing activity against several synthetic pyrethroids and are a major factor in the natural tolerance to these compounds.

The higher susceptibility of chrysopid adults to deltamethrin was reflected in significantly higher mortality in regard to the control, and furthermore, in a significant reproductive dysfunction. Low oviposition and viability of eggs with deltamethrin was because of the mode of action of pyrethroids, which act functionally on the voltage-sensitive sodium channel (Sattelle and Yamamoto, 1988). These compounds induce a shock effect in individuals that causes muscular convulsions and results in the disruption of physiological functions, such as reproduction, eventually leading to death (Bigler and Waldburger, 1994; Amarasekare and Shearer, 2013).

Regarding the novel insecticide sulfoxaflor, which acts to disturb the nervous system as a nicotinic acetylcholine receptor (nAChR) agonist (IRAC, 2012), no previous studies have evaluated the side effects in *C. carnea*. Nevertheless, compared with results with other insecticides with the same mode of action such as imidacloprid, the adult mortality at 3 d was similar (66.6%) (Huerta et al., 2003) to that obtained with sulfoxaflor (56.7%) in the present study. The developmental stage had again a relevant

Table 3
Effects on development, mortality and reproduction in *Adalia bipunctata* when L_4 larvae were exposed to fresh residues of insecticides on a glass surface, using the maximum field recommended concentration (MFRC) for horticultural crops.

Treatment	MFRC (mg a.i. L^{-1})	Pupation (%)[a]	Adult emergence (%)[b]	Cumulative mortality (%)[c]	Fecundity (eggs/♀ $week^{-1}$)[d]	Fertility (%)[e]	*E* (IOBC Cat.)[f]
Control	–	100.00 ± 0.00 a	100.00 ± 0.00 a	0.00 ± 0.00 a	79.15 ± 11.73 a	58.38 ± 1.84 a	–
Flonicamid	60	100.00 ± 0.00 a	100.00 ± 0.00 a	0.00 ± 0.00 a	103.64 ± 9.84 a	48.07 ± 3.56 a	−30.94 (1)
Flubendiamide	60	100.00 ± 0.00 a	100.00 ± 0.00 a	0.00 ± 0.00 a	105.00 ± 15.78 a	52.69 ± 3.91 a	−32.66 (1)
Metaflumizone	240	97.62 ± 2.38 a	92.86 ± 3.20 a	9.53 ± 3.01 a	63.80 ± 3.10 a	59.88 ± 7.74 a	27.08 (1)
Spirotetramat	75	100.00 ± 0.00 a	97.62 ± 2.38 a	2.38 ± 2.38 a	102.12 ± 14.20 a	47.00 ± 3.73 a	−25.95 (1)
Sulfoxaflor	63.6	7.14 ± 4.88 b	0.00 ± 0.00 b	100.00 ± 0.00 b	–	–	100.00 (4)
Deltamethrin	12.45	0.00 ± 0.00 b	–	100.00 ± 0.00 b	–	–	100.00 (4)

Data (mean ± SE) followed by different letters in the same column significantly differed (Kruskal–Wallis, Mann–Whitney, $P < 0.05$) for pupation, adult emergence and cumulative mortality.
Data (mean ± SE) followed by the same letter in the same column did not significantly differ (ANOVA, $P < 0.05$) for fecundity and fertility.
a.i. = active ingredient. *E* = total effect, according to Overmeer and Van Zon (1982).
[a] Percentage of formed pupae compared with the total number of treated larvae.
[b] Percentage of emerged adults compared with the number of formed pupae.
[c] Percentage of dead larvae, pupae and adults that failed to moult.
[d] Number of eggs laid per female measured 4–6 d after the emergence of adults.
[e] Percentage of hatched eggs versus laid eggs.
[f] IOBC toxicity category for laboratory tests based on the total effect caused by each insecticide: (1) harmless (<30%); (2) slightly harmful (30–79%); (3) moderately harmful (80–99%); and (4) harmful (>99%).

Table 4
Effects on mortality and reproduction in *Adalia bipunctata* when adults were exposed to fresh residues of insecticides on a glass surface, using the maximum field recommended concentration (MFRC) for horticultural crops.

Treatment	MFRC (mg a.i. L^{-1})	Adult mortality at 3 d (%)	Fecundity (eggs/♀ $week^{-1}$)[a]	Fertility (%)[b]	*E* (IOBC Cat.)[c]
Control	–	1.43 ± 1.43 a	81.08 ± 6.24 a	67.81 ± 6.60 a	–
Flonicamid	60	10.00 ± 3.64 ab	75.58 ± 11.25 a	57.39 ± 2.43 a	14.88 (1)
Flubendiamide	60	14.29 ± 4.52 ab	85.23 ± 13.91 a	50.30 ± 5.64 a	8.60 (1)
Metaflumizone	240	10.00 ± 3.64 ab	97.91 ± 17.98 a	59.95 ± 3.72 a	−10.26 (1)
Spirotetramat	75	10.00 ± 7.00 ab	94.69 ± 5.92 a	44.27 ± 10.60 a	−6.64 (1)
Sulfoxaflor	63.6	22.86 ± 6.93 b	101.83 ± 28.61 a	62.91 ± 3.32 a	1.71 (1)
Deltamethrin	12.45	100.00 ± 0.00 c	–	–	100.00 (4)

Data (mean ± SE) followed by different letters in the same column significantly differed (ANOVA, LSD, $P < 0.05$).
a.i. = active ingredient. *E* = total effect, according to Overmeer and Van Zon (1982).
[a] Number of eggs laid per female measured 4–6 d after the emergence of adults.
[b] Percentage of hatched eggs versus laid eggs.
[c] IOBC toxicity category for laboratory tests based on the total effect caused by each insecticide: (1) harmless (<30%); (2) slightly harmful (30–79%); (3) moderately harmful (80–99%); and (4) harmful (>99%).

influence on pesticide effects, because in sulfoxaflor exposure to L_3 larvae this compound proved to be harmless.

Concerning the coccinellid *A. bipunctata*, the susceptibility to insecticides was higher in L_4 larvae than in adults, the opposite of that observed for the chrysopid *C. carnea*. This likely was due to their closer adhesion to the substrate with anal pads and their higher foraging activity, both of which increase the likelihood of contact with a contaminated surface (Jalali et al., 2009). In a qualitative sense, the toxicity of the evaluated compounds was similar to both natural enemies because for *A. bipunctata* sulfoxaflor and deltamethrin were again the most damaging active ingredients. However, in a quantitative sense, because the cumulative larval mortality reached 100%, and thus, no adults emerged in any treatment, both compounds were substantially more toxic to *A. bipunctata*. According to the literature, the same toxic effects were observed with insecticides that had the same mode of action, such as imidacloprid and thiacloprid (IRAC MoA: 4) and lambda-cyhalothrin (IRAC MoA: 3), killing both larvae and adults (Jalali et al., 2009; Jansen, 2010). In a study by Olszak (1999) with different stages of *A. bipunctata*, pyrethroids were the most toxic compounds. In the adult bioassay, sulfoxaflor was harmless, as were the rest of compounds, except for deltamethrin, which caused 100% mortality after 3 d. This result was similar to that of Jalali et al. (2009), who found that adults were threefold more susceptible to lambda-cyhalothrin (pyrethroid, IRAC MoA: 3) than to imidacloprid (neonicotinoid, IRAC MoA: 4).

The effects of the insecticides flonicamid, flubendiamide, metaflumizone and spirotetramat on *C. carnea* and *A. bipunctata* were not comparable to those of sulfoxaflor and deltamethrin. A reasonable explanation to their harmless behavior can be found in the selectivity and mode of action of these compounds, because they are active mainly by ingestion (Tomlin, 2009), while the experiments evaluated the residual contact effects. For example, the selective homopteran feeding blocker flonicamid was found previously to be innocuous to L_4 larvae and adults of *A. bipunctata* even at a concentration ten-fold higher than recommended (Jalali et al., 2009). No literature references on the effects of flubendiamide, metaflumizone and spirotetramat on these BCAs were found, so the results obtained in this study will clarify the possibilities for combined use of both pest control strategies. Pesticide compatibility with biological control agents is a major concern in IPM and knowledge about the activity of insecticides toward the pests, the non-target insects, and the environment is a necessity (Stark et al., 2004). The present work studies the physiological selectivity of the tested insecticides in laboratory, but further research is required to determine the ecological selectivity. The use of selective pesticides at recommended concentrations, and spatio-temporal separation of pesticide applications and natural enemies

when possible, are main factors for integrating natural enemies with pesticides in pest management programs (Ruberson et al., 1998).

5. Conclusions

In conclusion, flonicamid, flubendiamide, metaflumizone and spirotetramat were nontoxic to last instar larvae and adults of *C. carnea* and *A. bipunctata*. These insecticides are good candidates to be incorporated into IPM programs in combination with these BCAs for the control of specific greenhouse pests, such as aphids, caterpillars, whiteflies and scales. By contrast, sulfoxaflor was slightly toxic to adults of *C. carnea* and was highly toxic to fourth instar larvae of *A. bipunctata*. Deltamethrin was the most toxic compound to larvae and adults of both natural enemies. Therefore, the use of deltamethrin and sulfoxaflor should be considered when releasing *C. carnea* and *A. bipunctata* as part of an IPM strategy.

The degree of toxicity of pesticides is influenced by the exposure method and the life stage, among others. Therefore, additional laboratory studies that assess more sublethal effects and field studies are needed to fully understand the selectivity of the tested insecticides to *C. carnea* and *A. bipunctata*, specially those that were toxic in laboratory.

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